COMPARATIVE STUDIES ON RESPIRATION XXII.

THE EFFECT OF LACTIC ACID ON THE RESPIRATION OF WHEAT

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Respiration in plants and animals involves a continuous series of linked reactions, of oxidative character, the end products being carbon dioxide and water. The reaction proceeds by stages, and though the actual steps may be unknown, many substances have been suggested as intermediate products. The hypothesis that the introduction into a respiring system, from without, of an excess of any one of the substances which are supposed to be intermediate stages in the metabolism, should accelerate the rate of production of carbon dioxide, forms a good basis of trial on which to test these suggested substances. On account of its frequent occurrence and apparent importance in animal metabolism, lactic acid was chosen as the sub ect of these experiments.

In dealing with animals, the effect of muscular action on the rate of production of carbon dioxide has to be taken into account. In plant material there are no such complications; it is easy to secure seedlings at a stage when the root system is well developed while the shoot has not yet begun to show green. With long roots and abundance of root hairs there can be no question as to the successful penetration of the reagent, which is an important point. Wheat was accordingly chosen as the material for these experiments. It was germinated with aseptic precautions, and used when the roots were about two inches long and well supplied with root hairs.

The method used for studying the respiration was that described by Osterhout,[1] using phenolsulphonphthalein as indicator. The normal rate of respiration (in distilled water) was taken as the reciprocal of the time required to change the indicator from pH 7.36 to pH 7.09, and was expressed as 100 percent. The actual time varied from 30 seconds to 1 minute, according to the age of the seedlings.

After taking the normal rate of respiration, the machine was stopped and a solution of lactic acid in distilled water was substituted for the distilled water in the flask containing the seedlings. The same volume, usually 100 cc., was used in each case. The first effect was a depression in the rate of respiration, due to the necessity of saturating with carbon dioxide the volume of fresh liquid introduced into the closed system. This "satu-

[1] Osterhout, W. J. V. Jour. Gen. Physiol. 1 : 17–22. 1918.

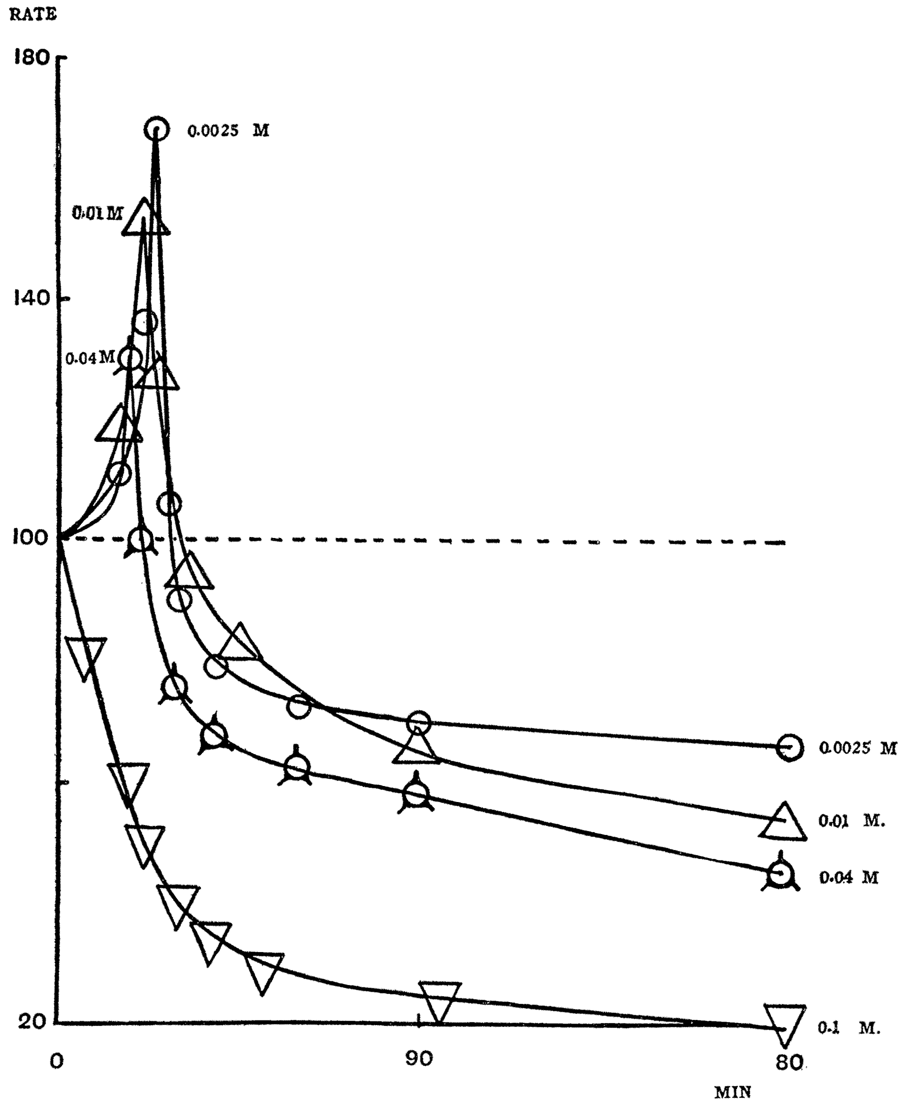

FIG. 1. Curves showing the rate of respiration of wheat seedlings (expressed as percentage of the normal rate), in lactic acid 0.0025M, 0.01M, 0.04M, and 0.1M. The normal rate (which is taken as 100 percent) is the reciprocal of the time required to change the indicator from pH 7.36 to pH 7.09 (usually from 30 to 60 seconds, according to the age of the material). The curve with 0.0025M lactic acid represents the mean of three experiments; probable error of the mean, less than 5 percent of the mean (after the maximum, less than 2 percent of the mean). The curve with 0.01M acid is the mean of two experiments; probable error of the mean, less than 3 percent of the mean. The curve with 0.04M acid represents a single typical experiment. The curve with 0.1M is the mean of five experiments; probable error of the mean, less than 10 percent of the mean.

ration effect" was determined for distilled water for each individual experiment, and allowance was made for this in drawing the curve. In no case did this preliminary lag in distilled water endure for more than 10 minutes, and it was shown, by introducing the lactic acid into the system in a separate tube, that it had no more buffer effect than the same volume of distilled water.

The lactic acid was used in the following concentrations: $0.0025M$, $0.005M$, $0.01M$, $0.02M$, $0.04M$, $0.1M$, $0.2M$, $0.5M$, and $2M$. This range was sufficiently close to give a good series of curves.

With lactic acid $0.0025M$, the first effect was a rise in the rate of respiration, which reached a maximum of 168 percent in 24 minutes after beginning the exposure to the acid. In 27 minutes the rate reached normal again, and then proceeded to fall below it. In 2 hours the rate had fallen to 68 percent.

With a concentration of $0.01M$, a maximum of 153 percent was reached in 21 minutes from the first exposure. The rate remained above normal for 30 minutes, and then fell to 61 percent in 2 hours.

With $0.04M$ lactic acid, the initial rise was smaller, and the time above normal was less. Thus, the maximum of 130 percent was reached in 18 minutes, and the rate returned to normal in 21 minutes. After 2 hours the rate was 55 percent.

With $0.1M$ lactic acid, there was no rise apparent. The rate fell steadily, at first rapidly and then more slowly, reaching 40 percent in 30 minutes, 28 percent in one hour, and 24 percent in two hours. After 4 hours the rate was reduced to 16 percent.

With intermediate strengths the results were intermediate and similar to those given. The figures represent the mean of several closely agreeing experiments.

In order to determine whether the osmotic pressure or the pH value of the lactic acid was contributing to the results obtained, experiments were made with sulphuric acid of the same pH value (about pH 3) as the $0.1M$ lactic acid. It was found that the sulphuric acid had no more effect than the same volume of distilled water. In the same way a 3 percent solution of dextrose, which was known to have a greater osmotic pressure than the $0.1M$ lactic acid, was found to have very little more effect than distilled water: and the "saturation effect" lasted only a few minutes longer. It was thus reasonably certain that the observed results were really due to some specific action of the lactic acid.

Recovery experiments were made with the $0.1M$, $0.2M$, and $2M$ acid. In all cases the seeds recovered completely on removal to distilled water, if sufficient time was allowed. Recovery was possible even if the rate had been reduced to 25 percent of the normal.

In these experiments there was no evidence of any permanent increase in the rate of production of carbon dioxide. This would seem to indicate

that in the case of wheat seedlings lactic acid is not an important stage in the normal metabolism. This is interesting in view of its apparent importance in the animal metabolism, and further experiments on animal tissues by this method should prove valuable.

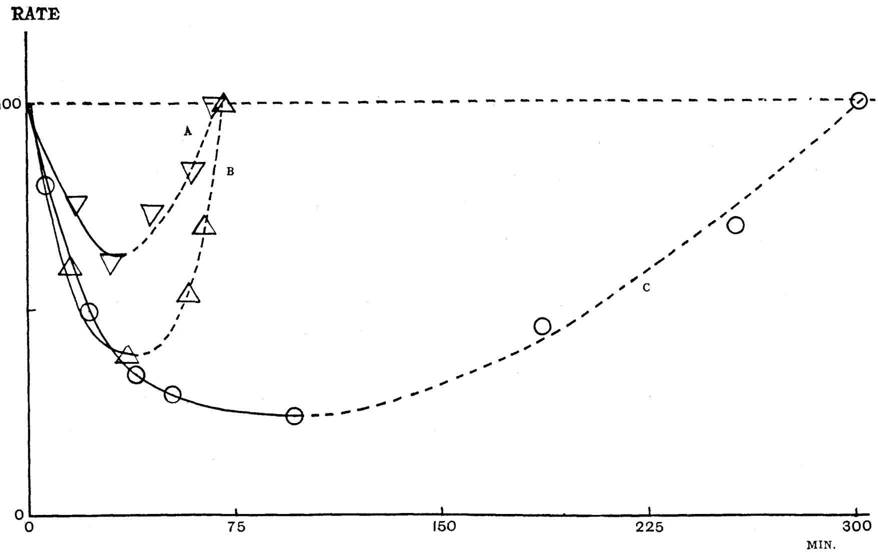

FIG. 2. Curves showing recovery from exposure to lactic acid. Normal rate as in figure 1. Solid line represents respiration in lactic acid; broken line, respiration in distilled water. Curve A in 0.2M acid, curves B and C in 0.1M acid. Each represents a single typical experiment.

Conclusions

1. In high dilutions, such as 0.0025M, lactic acid first accelerates and then depresses the rate of production of carbon dioxide by wheat seedlings.

2. As the concentration of the acid increases, the preliminary rise in rate becomes less marked, till a concentration is reached when the rate begins to fall at once.

3. Even if the rate has been rapidly reduced to 25 percent of the normal by 2M lactic acid, recovery is possible and appears to be complete.

4. The observed effects are due not merely to osmotic pressure or to acidity, but to some specific action of the lactic acid.

Since there is no permanent increase in the rate of production of carbon dioxide, as would be expected on the hypothesis that lactic acid is a stage in the metabolism of wheat, it may be concluded that lactic acid is not, in this case, an important intermediate substance.

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